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The fungicide thiram induced hepatic and renal injuries in domestic pigeons (Columba livia domestica)

Thiram induced liver and kidney perturbations in pigeon

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Abstract

Aim: Prolonged occupational exposure and the extensive agricultural use of thiram may affect animal and human health. The aim of this study is to investigate the effects of the dithiocarbamate fungicide thiram on hepatic and renal functions of domestic pigeons.

Material and Methods: Twenty-four (24) pigeons (Columba livia domestica) weighing 200 250g were divided into three (3) equal groups as follows: (CT) served as control, T, t groups were administrated orally 5 and 10 mg/Kg/day of thiram, respectively, for ten weeks. Each group was subdivided into four males and four females. In addition to the histopathological study of liver and kidney, levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), Gamma-glutamyl transferase (GGT), total bilirubin, total proteins, total cholesterol, triglycerides, glucose, uric acid and creatinine were evaluated.

Results: The obtained results indicated a significant increase in ALT, ALP and GGT activities with a slight increase in bilirubin, total proteins and glucose concentrations. However, total cholesterol and triglycerides were significantly elevated. The augmentations of uric acid and creatinine levels were dose-dependent. The liver and kidney somatic indexes were remarkably higher in the thiram-treated pigeons. Histological examination revealed hepatic architectural disorganization, lymphocyte infiltrations, accompanied by structural degradations of the renal parenchyma, glomeruli wall and tubules.

Discussion: In the present study, exposure of domestic pigeons (Columba livia) to thiram at 5 and 10 mg/Kg/day resulted in certain biochemical disorders. Hence, the determination of liver and kidney markers in pigeons exposed to thiram can give some indication of the general health status of birds. Oral exposure to thiram at 5 and 10 mg/Kg/day induced liver and renal perturbations.

Keywords

Thiram; Columba Livia Domestica; Liver; Kidney; Histology

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Introduction

Dithiocarbamate fungicides constitute a large group of used pesticides; due to low cost, good efficacy and a broad spectrum of antifungal activity. They are already used in agriculture and medicine. Thiram (tetramethyl thiuram disulfide: TMTD) is a protective dithiocarbamate fungicide used as a foliar treatment of fruits, vegetables, and ornamentals; it is applied to protect seeds before planting. Also, it is used as a rodent repellent to protect crops [1]. Thiram can disperse in the environment as an oxidation product of the fungicides, ziram, and ferbam. In spite of the extensive use, thiram has poor solubility in water (30 ppm at 25 °C); this makes it immobile in soils rich in clay and organic matter content. Thiram incorporation into the food chain could harm animal and human beings [2]. It exhibits low to moderate acute toxicity via the oral and dermal ways [3]. Chronic exposure has been found to cause adverse reproductive and developmental effects [4].TMTD is known as an inducer of allergic contact dermatitis and as an inhibitor of angiogenesis [5].Thiram residues in combination with nitrite could lead to carcinogenic nitrosamines. The neurotoxic, cytotoxic, genotoxic and mutagenic effects of thiram have already been reported [6]. Mainly metabolized in the liver, thiram cause some toxic effects on this organ, as indicated by hepatic enlargement, dysfunction, hepatitis, degenerative changes, and focal necrosis are reported[7].It can be metabolized to dimethyl dithiocarbamate (DMDC), carbon disulfide (CS2) and other products that had incriminated to inhibit microsomal enzymes [6].It has been reported that the disulfide groups present in thiram caused cells oxidation of proteins, peptides, and cofactors which provokes cytotoxicity [6]. Disulfide groups also can affect mitochondria and membrane lipid peroxidation, resulting in enhanced release of lactate dehydrogenases within the cells, increased serum aminotransferases activities and liver MDA contents, while it decreases serum alkaline phosphatase and hepatic antioxidant enzymes SOD, GSH-PX [8]. Samreen et al. [9] reported inhibition of all major antioxidant enzymes activities, elevated lipid and proteins' oxidation and hydrogen peroxide level in thiram treated erythrocytes. However, the same survey has shown a decrease in total sulfhydryl group content, glutathione hemoglobin oxidation levels and the release of free iron. Moreover, thiram may also cause damage to kidneys, in addition to disturbing glucocorticoid homeostasis [10].

Birds in the field may be exposed to thiram by ingesting treated seeds or by incidental ingestion of contaminated soil. Ecologically, high LC50 is not toxic to birds, but it could affect their reproductive mechanism [11,12]. Defined tibiae dyschondroplasia as a disease found in fast-growing strains of chickens, turkeys, and ducks.

Due to the lack of information on thiram toxic effects on birds, the present study aims to investigate the outcome of thiram on certain biochemical markers and histological profiles related to liver and renal functions of (Columba livia domestica) pigeon.

Material and Methods

Chemicals

Thiram (tetramethylthiuram disulfide, CAS 13726- 8), chemical purity 80%, 5.5% surface additives, and about 14.5% kaolin, was supplied by Sigma-Aldrich.

Animals

Twenty-four domestics pigeons (Columba livia domesica), (weighing 200250g) were captured locally in early February and were placed in metal cages (Animal house, Department of Biology, University of Skikda), with a free food (chick crumbs) and water intake. During ten weeks of experimental trial (from the last week of February to the first week of May), the pigeons were under a natural photoperiod, ambient temperature, and humidity. The birds were divided into three groups of 8 individuals (4 males and 4 females); the first group was the control (c), the second (d1) and the third (d2) orally received 5mg/Kg/day and 10mg/Kg/day of thiram, respectively. The doses administered in this experiment were chosen according to the LD50 of thiram to birds (670-2800 mg/Kg).

Sample preparation

At the end of the tenth week, the pigeons were sacrificed; then3 ml of blood was collected into heparinized tubes and centrifuged (3000 rpm. for 20 min) at 4°C. The livers and kidneys were immediately removed, weighed, and fixed in 10% formol solution for 24 hours, then passed in increasing alcohol grades for dehydration. After they cleared in toluene, the specimens were embedded in paraffin wax. Finally, sections (5µm thick) were mounted on diesterase phthalate xylene after they were stained with haematoxylin and Eosin.

Analytical procedures

The collected plasma was used for the measurement of alanine aminotransferase (ALT), glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin (TB), total proteins (TP), uric acid (UC) and creatinine using commercially available diagnostic kits supplied by Randox Laboratories (Ardmore, Northern Ireland, UK) using an automate CX9 (BECKMAN, Brea, CA, USA). Plasma glucose was estimated using assay kits supplied by Diamond Diagnostics. Total cholesterol (TC) and triglycerides (TG) were estimated by colorimetric methods using Randox reagents.

Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis was obtained using two-way analysis of variance (ANOVA) with multiple comparisons using Tukey's test at $p \leq 0.05$ using the Minitab version 19.

Ethical Approval:

Experiments on pigeons were carried out according to the national animal welfare and to the EU Directive N° 2010/63/EU on the protection of animals used for scientific purposes.

Results

None of the pigeons treated with thiram 80, at doses of 5 and 10 mg/ Kg/day have shown signs of morbidity or mortality during the study, but diarrhea and the decrease in food intake were observed along the experimental period.

Effects of treatment on body, liver and kidney weights

The results indicated that relative weights of livers and kidneys were increased (insignificantly) in the two sexes of pigeons treated with the two thiram doses compared to the control (Table 1), with no remarkable variations in total body weight between all groups

Effect of treatment on plasma biochemical parameters

Table 2 reports the mean values of various biochemical

Table 1. Mean ± SEM of liver, kidney and body weights (g) of control and thiram-treated pigeons after 10 weeks of experimental trial.

Groups	Control (c)		5 mg /Kg/D (d1)		10 mg /Kg/D (d2)	
	Males	Females	Males	Females	Males	Females
TBW	284±14.73	261±2.65	267±7.21	262±9	268±7.66	264±24.6
LW	3.56±0.29	3.66±0.33	3.76±0.17	4.04±0.94	3.8±0.48	4±0.33
KW	0.23±0.11	0.16±0.05	0.30±0.1	0.25±0.05	0.40±0.2	0.27±0.05

TBW: Total body weight; LW: Liver weight, KW: Kidney weight, n = 4.

Table 2. Mean ± SEM of plasma biochemical markers in control and thiram-treated pigeons after 10 weeks of experimental trial.

	Control		5mg /Kg/D		10 mg /Kg/D	
	Males	Females	Males	Females	Males	Females
ALP	80±13	95±17.3	341±57.6[a]	289±46.1[a]	446±83.3[b]	304±9.76[b]
GGT	129.4±6.58	125.4±6.4	179.7±7.58[a]	152±12.73[a]	201.6±9.2[b]	174±11.3[b]
ALT	18.8±5.38	18±65	42±15.7[a]	29.25±2.5	44±8.42[b]	38.61±1.79[b]
TB	2.6±0.2	2.6±0.2	10.3±1.25[a]	7.53±2.83	9.58±1.4[b]	7.94±2.56[b]
TP	24.6±32	28.2±4	29.5±8.64	30.08±6.7	42.9±5.38	45±28
TC	1.8±0.36	1.44±0.17	2.43±0.24	1.44±0.17	2.25±0.30	2.1±0.38[b]
TG	1.4±0.22	1.3±0.07	1.93±0.07	1.98±0.24[a]	2.14±0.37[b]	2.12±0.25[b]
Gl	2.50±0.09	2.34±0.46	2.85 ±0.07[a]	2.51±0.34	2.94±0.31[b]	2.67±0.23
UC	51.2±18	32±19	41.7±19.95	63±17.4	53±16	75.7±16.1[b]
Cr	5.2±1.8	6.08±0.4	4.01±0.8	6.1±0.5	5.2±1.2	6±1.8

GGT: Gamma-glutamyl transferase (IU/L), ALT: Alanine aminotransferase (IU/L), ALP: Alkaline phosphatase (IU/L), TB: total biluribin (mg//l); TP: total protein (g/l); TC: total cholesterol (g/l); TG: trigycerids (g/l); Gl: glucose (g/l); UC: uric acid (g/l); Cr: creatinine (g/l) ,
[a-b]; Significant difference at p≤0.05 was indicated by superscript letters, as analyzed by two-way ANOVA test (n= 4).

parameters of domestic pigeons Columba livia domestica treated with thiram at doses of 5 and 10 mg/ Kg/ day for 10 weeks.

Thiram induced a significant increase in hepatic enzyme activities (ALT, GGT, and ALP) in both male and female pigeons. However, plasma glucose and total proteins significantly increased with the increasing dose in both males and females. In term of lipid profile, the level of total cholesterol and triglycerides were higher in the treated groups increased significantly. Total triglycerides were higher in treated females. Concerning the renal function, Table 2 shows statistically more significant increases in acid uric and creatinine levels in the treated groups. It is interesting to note that the higher levels of acid uric and creatinine were among the treated females.

Effect of treatment on hepatic histology

The histological examination of the liver section of the control group showed normal healthy parenchyma. However, liver section of treated pigeons demonstrated necrotic and degenerative changes with congestions of the hepato-portal blood vessel, and inflammatory infiltrations (Figure 1).

Effect of treatment on kidney histology

Sections of the treated pigeons showed that the proximal and distal convoluted tubules were less condensed than those in the control group. A change in the structure of the renal glomerulus compared to the control with important changes in the renal parenchyma (decreased branching), depletion of the glomeruli wall and the presence of eutrophication in the parenchyma and in the glomeruli. Thiram at different concentrations showed a dilating effect on the renal tubules and disorganization of the capillary pellets of the glomeruli (Figure 2).

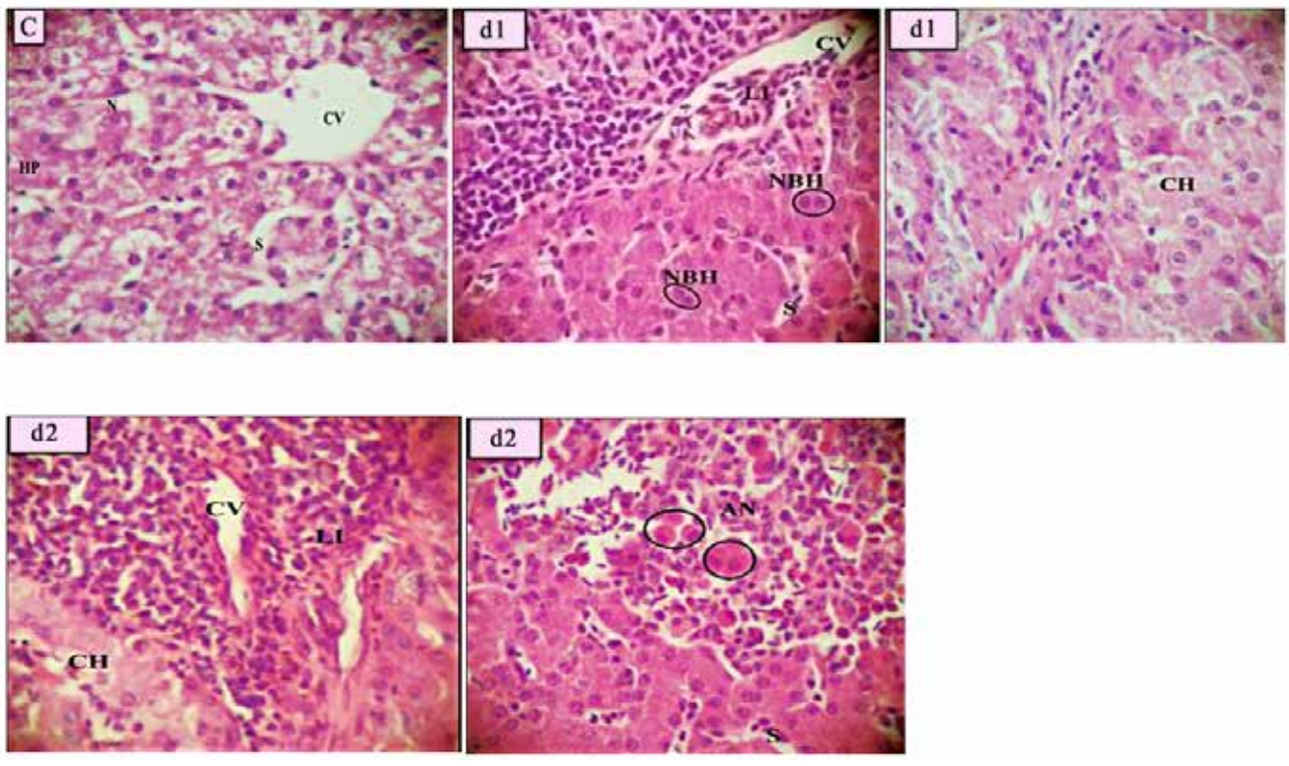

Figure 1. Microphotograph of liver tissue Section of (c) the control, (d1) 5mg /Kg/D of thiram (d2) 10mg /Kg/D of Thiram
Control group. CV: Centrilobular vein; N: nucleu; HP: hepatic parenchymaS: sinusoid
d1 group; CH: clarification of hepatocytes; CV: centrilobular vein; NBH: nuclei in binucleated hepatocytes; LI: lymphocytic infiltrations; S: sinusoid.
d2 group; AN: acidophilic nucleus; CV: centrilobular vein; LI: lymphocytic infiltrations; CH: clarification of hepatocytes; S:sinusoid.

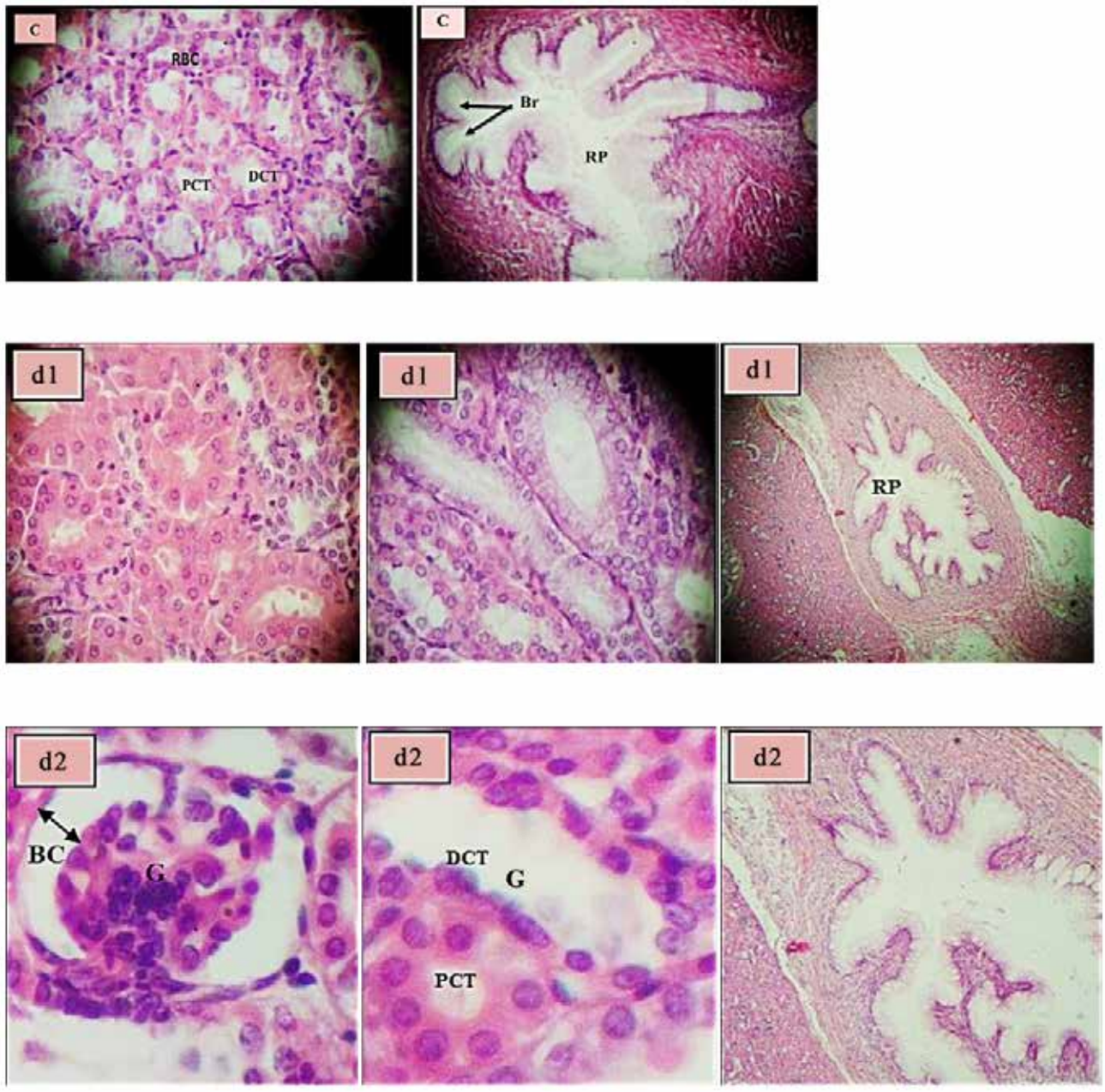

Figure 2. Microphotograph of kidney tissue of (c) the control pigeon showing healthy parenchyma with numerous and normal proximal and distal convoluted tubules; (d1) pigeons treated with 5mg/Kg/D of thiram; (d2) pigeons treated with 10mg/Kg/D of thiram. RBC: red bound cells; PCT: proximal convoluted tubules; DCT: distal convoluted tubules; Br: branching; RP: renal parenchyma; BC: Bowman capsule; G: glomeruli

Discussion

In the present study, exposure of domestic pigeons (Columba livia) to thiram at doses of 5 and 10 mg/Kg/day resulted in certain biochemical disorders. Hence, the determination of liver and kidney markers in pigeons exposed to thiram can give some indication of the general health status of the birds. Alanine aminotransferase and alkaline phosphatase have already been used to evaluate hepatic damage in clinical investigations [13]. The present study showed a significant increase in plasma ALT, GGT and ALP activities of pigeons after thiram exposure. These results are consistent with that recorded by Samreen [9], where thiram raised plasma ALT and AST and liver MDA, while they decreased in male broiler chicks. Moreover, an increase in serum ALP activity and liver antioxidant enzymes (SOD, G-Px) was observed in maneb and paraquat-treated rats [14], and as well as after treatment with carbofuran Clarias gariepinus[15]. Abd-Alrahman et al [16] have reported a higher ALT activity in the albino rats treated with difenoconazole, or diclofop-methyl alone, or in combination. All of these perturbations are related to liver damages, which is compatible with histopathological lesions that clearly showed degeneration and necrosis of the hepatic cells, accompanied by an increase in AST and ALT activities and confirm the pesticide induced liver injury. A slight increase in plasma total bilirubin may be associated with primary periportal necrosis as a result of liver malfunction. In this study, a slight increase in the concentrations of total proteins plasma confirms the thiram toxicity. It is known that proteins could preserve the organism from xenobiotics, parasites and infections [17].

Glucose is one of the important sources of energy for cell

functions and it is believed that glucose level might be used as an indicator of stress from pollution and from other physical factors. Actually, thiramtreatment at the dose of 5 and 10 mg/Kg/day increased serum glucose in a dose-dependent manner in Columba livia. Mekkawyet al. [18] indicated that the elevation of corticosteroid hormones and plasma catecholamines caused liver glycogenolysis, which lead to hyper-glycemia. Gluconeogenesis from amino acids also may be incremented in glucose elevation. Accordingly, an increase in glucose levels was recorded after exposure to thiram on freshwater fish (Cyprinus carpio) [19] and exposure to propineb on pigeons (Columba livia) [20]. In this study, serum creatinine levels were increased with increasing dose of thiram; this elevation may be associated with the impairment of the glomerular function and to damage to the renal tubules, which is in-line with the tubular histopathological studies. Serum cholesterol and triglycerides were significantly elevated, that may indicate certain changes in the hepatic cell permeability [21]. In fact, the increase in total cholesterol assessed in this study may be related to the blocking of hepatic bile ducts, causing, however, a decrease in cholesterol secretion into the duodenum [22].

The data from the histopathological study indicated alterations of thiram in the liver and kidney of pigeons of both sexes, as that of the biochemical markers' impairments. Destruction of the liver architecture, necrotic and degenerative changes with congestions of the hepatic portal blood vessel, and inflammatory infiltrations were observed in thiram-treated pigeons. Dithiocarbamates fungicides (DTCs) like thiram were toxic to the liver, kidney and testis [23]. Earlier, O¨zbay et al. [24] showed that maneb and zineb caused some pathological changes in the liver of mice indicated by vein congestion and mononuclear inflammatory cell infiltrations. Accordingly, the current histopathological investigation confirmed that thiram at different concentration induces harmful effects on renal architecture, where the proximal and distal convoluted tubules are less condensed, decreased branching of renal parenchyma, depletion of the glomeruli wall and the presence of eutrophication in both parenchyma and the glomeruli. Thus, thiram had dilated the renal tubules and disorganized the capillary pellets of the glomeruli. Guven et al. [25] reported that the kidneys of the propineb-treated rats showed oedema, cell degeneration, and irregularities in tubular structure. Furthermore, kidneys section of maneb-treated rats shows clearly dense hemorrhage, disappearance of epithelium cells around tubules, infiltration of inflammatory cells towards tubules' lumen and clear edema between tubules.

In conclusion, thiram modified liver and the kidney markers in domestic pigeons (Columba livia domestica).

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Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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